HUMAN BRAIN ORGANOID-ON-CHIP PLATFORM TO IMPROVE ORGANOID REPRODUCIBILITY AND SCALABILITY FOR PHARMACEUTICAL STUDIES

Héloïse Castiglione[1,2,3], Lucie Madrange[2,3], Camille Baquerre[1], Benoît G. C. Maisonneuve[1], Johan Renault[1], Frank Yates[2,3], Pierre-Antoine Vigneron[2,3,*], Jessica Rontard[1,*], Thibault Honegger[1]

[1] NETRI, 69007 Lyon, France, [2] SupBiotech, Ecole d'ingénieurs en Biotechnologies, 94800 Villejuif, France, [3] Service d'Etude des Prions et des Infections Atypiques (SEPIA), Institut François Jacob, Commissariat à l'Energie Atomique et aux Energies Alternatives (CEA), Université Paris Saclay, 92260 Fontenay-aux-Roses, France

BACKGROUND

Cerebral organoid + **Microfluidic device (NETRI)**

Cerebral organoids are emerging as relevant alternatives for modeling human brain cellular organization and development in 3D. To facilitate the use of organoids for large-scale drug screening, they require a gain of reproducibility and scalability (Castiglione *et al.*, 2022). To address this challenge, we have:

- Developed a Brain Organoid-on-a-Chip platform, combining cerebral organoid culture within a NETRI microfluidic device
- Optimized an on-chip culture protocol for cerebral organoids, enhancing their reproducibility
- Established a scoring system & a prediction algorithm, for quality control and toxicological evaluation of cortical organoids exposed to chemical compounds

Using this platform, we have initiated neurotoxicity evaluations of vanillin and biphenyl-2-ylamine.

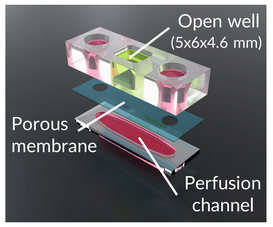
EXPERIMENTAL DESIGN

DUPLEX WELL MICROFLUIDIC DEVICE

Adapted to 3D cell culture:
Two compartments separated by a porous membrane:
- Open well for 3D culture
- Perfusion channel

Adapted to industrial transfer

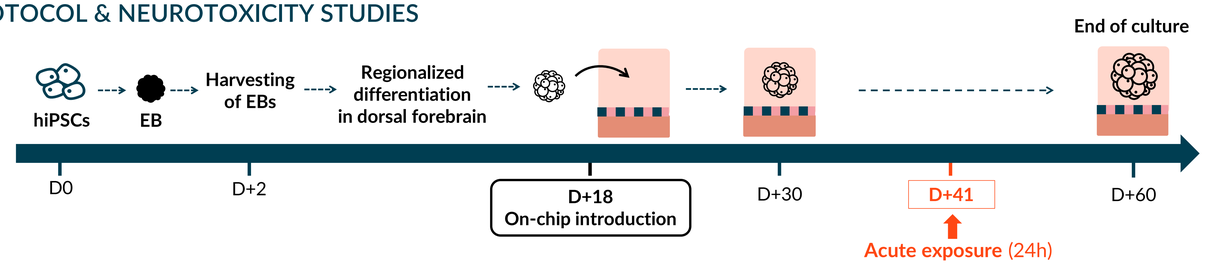
Pumpless



Duplex Well (©NETRI)

BRAIN ORGANOID-ON-CHIP PROTOCOL & NEUROTOXICITY STUDIES

Standardized on-chip protocol for cortical organoid culture
- Viability and expected morphology up to D+120
- Expected RNA expression levels at D+60
- Improved cytoarchitectural organization at D+60
- Anti-adherence protocol to limit organoid adherence onto membrane



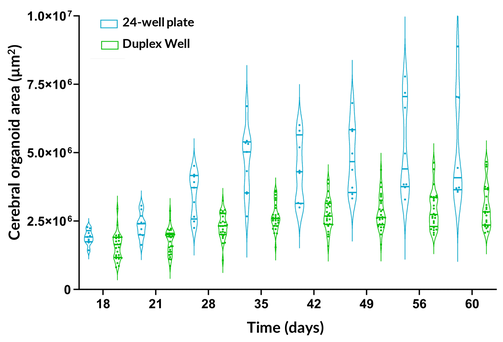
On-chip culture conditions and neurotoxicity evaluations.
Cortical organoid generation and culture protocol adapted from Xiang *et al.*, 2019; hiPSCs: human induced pluripotent stem cells, EB: embryoid body.

Vanillin (CAS n°121-33-5): 100, 1 000, 10 000 nM
Biphenyl-2-ylamine (CAS n°90-41-5): 20, 200, 2 000 µM *(meOH)*

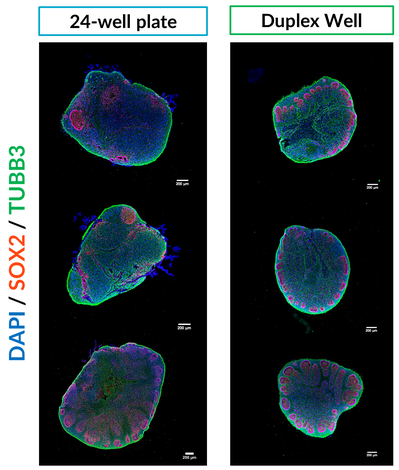
RESULTS

IMPROVED REPRODUCIBILITY ON-CHIP COMPARED WITH CONVENTIONAL SUPPORT

More reproducible sizes of cerebral organoids on-chip compared to controls cultured in 24-well plates
- Lower size dispersions for organoids on-chip compared with organoids cultured in 24-well plates → reduced variability
- Smaller average organoid sizes on-chip could be due to the culture in a constraint environment
- *Observed with two hiPSCs lines*



Cerebral organoid sizes and growth evolutions from D+18 until D+60 of culture, for on-chip organoids and control organoids cultured in 24-well plates. On-chip organoids exhibit more reproducible surface areas (24-well plate: n=9; Duplex Well: n=27).



Immunofluorescence staining of neural progenitors (SOX2) and neurons (TUBB3) in cerebral organoids after 60 days of culture. (Thunder microscope, Leica, objective 10X).

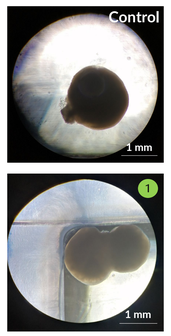
More reproducible cytoarchitectural organization in organoids on-chip than in 24-well plates

Rosettes with expected morphologies (oval shapes) & well-organized patterns (present along organoid borders

NEUROTOXICITY STUDIES USING THE BRAIN ORGANOID-ON-CHIP PLATFORM

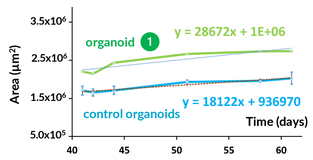
Example 1 : acute exposure (10 000 nM vanillin)

Optimal morphology



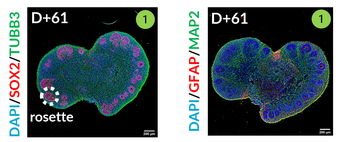
Morphology of cortical organoids at day 61: examples of an unexposed control organoid and vanillin-exposed organoid #1 (brightfield, 5X).

Similar growth profile compared to controls



Cortical organoids growth curves and slopes between exposure (D+41) and D+61 (controls: mean ±SEM, n=4).

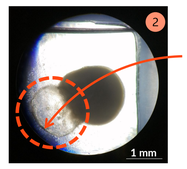
Expected cell types and optimal cytoarchitectural organization



Immunofluorescence staining of neural progenitors (SOX2), neurons (TUBB3, MAP2), and astrocytes (GFAP) (Thunder microscope, Leica, 20X).

Example 2 : acute exposure (2 000 µM biphenyl-2-ylamine)

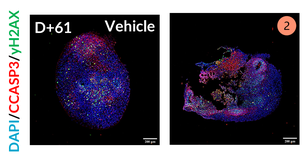
Altered morphology



presence of a large cyst *(> 25% of total surface area)*

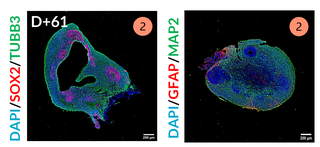
Morphology of cortical organoid #2 at 61 days of culture (brightfield, 5X, circle: cyst).

Higher apoptosis & DNA damage levels



Immunofluorescence staining of apoptosis (CCASP3) and DNA damage (γH2AX) markers (Thunder microscope, Leica, 20X).

Expected cell types with disorganized cytoarchitectures



Immunofluorescence staining of neural progenitors (SOX2), neurons (TUBB3, MAP2), and astrocytes (GFAP) (Thunder microscope, Leica, 20X).

- Altered pattern of rosettes
- Presence of a large necrotic core

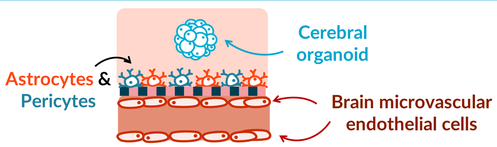
General conclusions using the scoring system & prediction algorithm

Vanillin exposures: no discernable impact on morphology, cytoarchitectures & viability

Biphenyl-2-ylamine exposures: altered morphology & disorganized cytoarchitectures in a dose-response manner

CONCLUSION & PERSPECTIVES

- Optimized cerebral organoid culture protocol on-chip
- Improved intra- and inter-batch reproducibility: in terms of size, growth profile and cytoarchitectural organization
- Brain Organoid-on-Chip platform + scoring system + prediction algorithm: adapted to neurotoxicity evaluations
- Evaluation of the neurotoxicity of other compounds to refine the prediction algorithm
- Modelling the blood-brain barrier
- Our Brain Organoid-on-Chip platform paves the way for drug screening and toxicological assessments



MPS WORLD SUMMIT
CONNECT, EXCHANGE, EDUCATE

Presented on 2024-06-10 - 14
[Seattle, Washington, USA]

CONTACT

www.netri.com
Phone: +33 4 78 23 08 66
Email: contact@netri.com

www.recherche.supbiotech.fr
Email: pierre-antoine.vigneron@supbiotech.fr